PhD THESES

INVESTIGATION OF THE MOLECULAR STRUCTURE, DISTRIBUTION AND FUNCTION OF THE HCN AND TASK CHANNELS IN THE CENTRAL NERVOUS SYSTEM

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INTRODUCTION

The cochlear nucleus has a fundamental role in the central processing of the auditory information, as the primary analysis of the action potential firing pattern carried by the auditory nerve begins here. It was a particularly important finding of the studies concerning the physiological functions of the cochlear nucleus that its projection neurones respond differently to the incoming stimuli, providing important contribution to the signal processing, integrating and transferring activity of the nucleus.

The most prominent projection neurones of the dorsal cochlear nucleus are the pyramidal cells. The pyramidal cells possess a triangle-shaped cell body whose diameter is 20-25 μm. They have an apical and a basal dendritic tree extending from the soma. The apical dendritic tree makes several contacts with the cochlear granule cells; while the basal dendrites are contacted with the primary acoustic nerve fibres via excitatory, glutamatergic synapses. Previous studies have demonstrated the ability of pyramidal cells to perform complex signal analysis. This feature is the consequence of their capability of producing several distinct firing patterns depending on their initial membrane potential and hence on the availability of the voltage-gated ionic channels for activation. The function and signal analysis of the pyramidal cells are entirely different from those of the bushy neurones, which are regarded as the other principal type of projection neurones of the cochlear nucleus. It as been indicated earlier that the highly specific activity pattern of the pyramidal cells is ensured by the presence and activity of rapidly inactivating, depolarisation-activated K^+ channels; slowly inactivating K^+ channels, persistent Na^+ channels and various types of voltage-gated Ca^{2+} channels (T-, P/Q-, L-, N- and R-type).

Unlike the bushy neurones, pyramidal cells show spontaneous activity, whose exact source is still unknown. Some authors believe that pyramidal cells have an intrinsic pacemaker activity, but others suggested that the spontaneous activity is just the consequence of the spontaneous firing of certain cells making synaptic contacts with the pyramidal neurones. Unfortunately, there are no experimental data at all about the presence and possible functions of the hyperpolarization-activated non-specific cationic current (termed I_h) in pyramidal cells, despite the fact that the channels producing I_h (hyperpolarization-activated cyclic nucleotide-gated or HCN channels) are responsible for the spontaneous activity of several structures (i.e. cells of the sinoatrial node). Although HCN channels have been noted in certain neurones of the auditory apparatus too, hitherto no specific experiments have been conducted to check for the presence of this current in the cases of the pyramidal neurones. In

the present work we performed a study to investigate the presence and possible source of the spontaneous activity of the pyramidal cells.

According to the most recent experimental data, the resting K^+-conductance, the resting membrane potential, input resistance and in the cases of excitable membranes the excitability of the surface membrane are all determined by the TASK-channels (TWIK-releated acid sensitive K^+ channel; TWIK: twin-pore in weak inward rectifier K^+ channel). TASK channels belong to the newly discovered two-pore domain K^+ channel family. As significant amounts of TASK-specific mRNA have been reported in various parts of the central auditory system, including the rat cochlear nucleus; it seemed logical to assume that these ionic channels may have important roles in the function of the principal projection neurones of the cochlear nucleus. Prior to the detailed and specific examinations concerning the presence and function of the TASK channels in certain neurones of cochlear nucleus, it was necessary to check whether glial cells expressed these channels; particularly in the light of the possible interactions between glial cells and neurones (and all of the factors altering them), which can significantly modify the functions of nerve cells. We had an opportunity to study the distribution of TASK channels in the human central nervous system, providing data about the localisation of TASK channels in the human brain for the first time in the literature. This work was conducted on the human cerebellum, as the consequence of its relatively easy identification and simple histological structure. The use of cerebellum was further justified by the fact that there are well-known similarities between the cerebellum and cochlear nuclei. There were already data available in the literature about the distribution of certain types of TASK channels in the rat cerebellum, hence the employment of cerebellar tissue samples was particularly helpful as it allowed the thorough comparison of the TASK expression of the rat and human brain.

In the present work answers were sought for the following questions:

1. Is it possible to detect spontaneous activity of the pyramidal neurones in brain slices prepared from the cochlear nucleus of the rat?
2. Do pyramidal neurones express HCN channels, which often provide the ability of spontaneous activity in other structures? If so, what are the electrophysiological and pharmacological properties of these channels and the current passing through them?
3. Is there any correlation between the existence of HCN channels and the spontaneous activity of the pyramidal neurones?

4. Is it possible to provide information about the putative subunit composition of the HCN channels expressed by the pyramidal cells of the cochlear nucleus?
5. Is it possible to detect the presence of TASK-1, TASK-2 and TASK-3 channels in astrocyte cultures prepared form the cochlear nucleus? Is it possible to confirm the immunocytochemical data by means of functional measurements (i.e. detecting the ionic current passing through TASK-channels)?
6. Is there any correlation between the expressions of TASK channels and the source of the astrocytes (cochlear nucleus, hippocampus, cerebellum)?
7. Is there any difference between the expression patterns of the investigated TASK channels in GluT- and GluR-type astrocytes?
8. Are TASK channels present in the human cerebellum? If so what is the distribution pattern of these channels?
9. Is it possible to detect any difference between the distribution patterns of the TASK-channels in the human and rat cerebellum?

MATERIALS AND METHODS

Electrophysiological measurements were carried out on 100-200 μm thick brain slices isolated from 8-14-day-old rats as well as on cell cultures prepared after the enzymatic isolation of astrocytes from 3-8-day-old Wistar rats. The ionic currents and changes of the membrane potential were recorded by employing the whole-cell configuration of the patch-clamp technique. To ensure that only pyramidal cells were investigated in the present study a fluorescent dye (Lucifer Yellow) was present in the pipette solution. The experiments conducted on brain slices were performed by using either Nomarski-optics or (at the end of the measurements, when the investigated cell was filled with Lucifer yellow) epifluorescent illumination.

The possible subunit composition of the HCN channels expressed by the isolated pyramidal cells was determined by employing HCN1-, HCN2- and HCN4-specific immunolabelling.

Expression of TASK-1, TASK-2 and TASK-3 channels was investigated on astrocyte culture and 4 μm thick tissue sections. The astrocyte cultures were 4-5-day-old and showed ~70-80% confluence when TASK specific immunolabelling was performed. The identification of glial cells was achieved by demonstrating the presence of GFAP (glial fibrillary acidic protein) specific immunoreactions. Both GFAP- and TASK-specific immunoreactions were visualized by applying fluorescent fluorophor (FITC) conjugated secondary antibodies. The immunoreactions of astrocyte cultures were recorded by using fluorescent or confocal laser microscopy. Western blotting and RT-PCR confirmed the results of immunocytochemical reactions.

To investigate the presence of TASK channels in the endoplasmic reticulum, the cultured astrocytes were occasionally transfected with the pDsRed2-ER vector. 24 hrs after the transfection the cells were fixed in formaldehyde then TASK specific immunolabelling was carried out. To quantify the co-localisation, a pixel analysis was performed where the percentage of pixels showing both green (TASK) and red (DsRed2-ER) positivity was determined, relative to the number of all green pixels.

When tissue sections from rat or human cerebellum were investigated, non-fluorescent immunohistochemistry was used. Formaldehyde-fixed, paraffin-embedded sections were prepared from the human cerebellum, while in the case of the rat cerebellum both formaldehyde-fixed (24 hrs) and frozen (in liquid nitrogen) sections were employed. (The sections were 4 μm thick in every case.) The formaldehyde-fixed sections were subjected to

antigen retrieval (by using either pronase treatment or incubating the tissue specimen in 0.01 M TRIS-buffer in a microwave oven).

In some cases double immunolabelling was performed on the cerebellar histology specimens (glia or neurone specific reactions were combined with the TASK specific labelling). In some instances counterstaining (haematoxylin) was also applied.

RESULTS

1. *Significance of the HCN current of the pyramidal cells of the dorsal cochlear nucleus in shaping their characteristic activity pattern*

1.1. General characterisation of the HCN current

The electrophysiological investigation of the pyramidal cells was performed by using microelectrodes filled with a Lucifer Yellow containing pipette solution. During the measurements the fluorescent dye diffused into the cell, making the shape of the cell body and a significant portion of the dendritic arborisation visible after the measurements. Pyramidal cells were identified on the basis of the large, triangular cell body (20-25 μm) giving rise to the apical and basal dendritic trees as well as the axon.

During the experiments 2 s long hyperpolarizing stimuli were delivered between -70 and -140 mV from a holding potential of -60 mV. In response to the hyperpolarizing stimuli pyramidal cells produced a slowly activating inward current showing no obvious inactivation tendency. The reversal potential of the current was determined by using the extrapolation method and it proved to be -32 ± 3 mV (mean ± SEM, n = 6). The slow activation of the current, the lack of inactivation along with its rather positive reversal potential suggested that this current corresponded to the hyperpolarization-activated non-specific cationic current (I_h) whose genesis is ensured by the activation of the HCN channels.

The half activation voltage of I_h was -99 ± 1 mV with a slope factor of 10.9 ± 0.4 mV (n = 27). The activation time constants describing the activation kinetics were determined by fitting the activation phase of the h-current. The activation of I_h could be best described by using a double exponential function. The actual values of the activation time constants varied between 150-300 ms (τ_1) and 800-1500 ms (τ_2) depending on the amplitude of the hyperpolarizing stimuli.

1.2. Pharmacological test of the HCN-current and its relationship with the spontaneous activity of pyramidal cells

The hyperpolarization-activated current is known to be sensitive to the extracellular application of CsCl, hence we investigated its effect on the h-current expressed by the pyramidal cells. 1 mM CsCl reduced the amplitude of I_h by 85 ± 9 %; (n = 12; -140 mV). The presence of 5 mM Cs^+ was also tested resulting in a similar blocking effect (87 ± 7%, n = 5).

A significant portion of the pyramidal cells showed spontaneous activity (69 cells out of 98) in our experiments. As the h-current is responsible for the intrinsic activity of a number of cell types, we determined the effect of the h-current blocking CsCl on the spontaneous activity of the pyramidal cells. The intrinsic firing was detected in the current-clamp configuration without applying external stimuli, in control extracellular solution first then in the presence of 1-5 mM CsCl. When CsCl was present the slow depolarization preceding the individual action potentials became shallower, and thus the frequency of the action potential firing decreased to approximately 26% of the control activity (n = 5).

Since CsCl is capable of inhibiting other ionic currents as well besides I_h, the effect of a selective h-current inhibitor (ZD7288) was investigated too. In the presence of 10 μM ZD7288 the amplitude of hyperpolarization-activated current and the spontaneous firing frequency were reduced to approximately 20% of the control (n = 5).

1.3. HCN-subunit composition of the pyramidal neurones

Besides the electrophysiological characterization of the pyramidal h-current, immunocytochemical experiments were also conducted to check for the putative subunit composition of the HCN channels responsible for the genesis of I_h. In these experiments primary antibodies targeting HCN1, HCN2 and HCN4 subunits were applied. Although the characteristic morphological features of the pyramidal neurones are present even after enzymatic isolation, the HCN specific immunolabelling was often complemented by anti-NSE (neurone specific enolase) specific immunostaining. The results of the immunocytochemistry experiments clearly indicated that the channels responsible for the genesis of the h-current contained all investigated types of HCN subunits (HCN1, HCN2 and HCN4).

2. *Investigation of the presence and distribution of TASK-channels in the rat and human central nervous system*

2.1. Distribution of TASK-immunoreactivity in the rat cerebellum

In the frame of the immunohistochemical investigation of formaldehyde fixed, paraffin-embedded cerebellar tissue sections of the rat, the presence of TASK-1, TASK-2 and TASK-3 channel subunits was detected. The Purkinje-cells and the pia mater showed particularly strong TASK-1 positivity, but positive labelling could also be observed in the molecular and granule layers. The distribution of TASK-2 immunopositivity was almost the same as the TASK-1 specific reaction, but its intensity was somewhat weaker. Purkinje-cells

possessed pronounced TASK-3 positivity, and the molecular and granule layers proved to be positive as well. No TASK-3-specific labelling was found on the pia mater. Moreover, strong TASK-3 labelling could be detected in the white matter of cerebellum too.

In frozen sections double immunolabelling was performed to check for the presence of TASK expression of the astrocytes. In these experiments the glia specific immunolabelling was achieved by using anti-GFAP primary antibodies. The astrocytes proved to be positive for all investigated types of TASK channels (TASK-1, TASK-2 and TASK-3).

2.2. Presence of TASK-immunoreactivity in astrocyte cultures

The TASK-1, TASK-2 and TASK-3-immunopositivities of cerebellar astrocytes were confirmed by immunocytochemistry performed on primary astrocyte cultures. On the basis of the appropriate morphological features and GFAP-positivity exhibited by the astrocytes, both known types of astrocytes (GluT and GluR) could be identified. GluR and GluT cells showed different TASK-1 positivity: GluT astrocytes exhibited strong TASK-1-immunolabelling, while GluR cells showed somewhat weaker (but nevertheless present) TASK-1 expression. The TASK-2 and TASK-3 positivity was equally strong in both types of astrocytes, but generally weaker than the intensity of the TASK-1 immunolabelling. Experiments conducted on astrocyte cultures prepared from the hippocampus and cochlear nucleus gave identical results.

The results of the immunocytochemical investigations were confirmed by using molecular biology techniques too. In these experiments the presence of TASK-1, TASK-2 and TASK-3 specific mRNA was demonstrated by employing RT-PCR, while the expression of the appropriate protein products was detected by Western blotting.

The presence of glial TASK channel expression was confirmed by functional measurements too. The current amplitude produced in response to depolarizing stimuli was reduced to $63 \pm 4\%$ of the control (pH = 7.2) when acidic (pH = 5.2) extracellular solution was applied (n = 12). Similar observations were made when the experiments were conducted on hippocampal or cochlear astrocyte cultures.

2.3. Expression of TASK-channels in the endoplasmic reticulum of the astrocytes

The intracellular distribution of the TASK-channel subunits was also investigated in the present work by using astrocyte cultures. The results of confocal microscopy indicated that TASK channels were present intracellularly, perhaps in the membrane of the endoplasmic reticulum (ER). In order to prove this possibility, astrocyte cultures were transfected with the

pDsRed2-ER plasmid, whose protein product specifically targets the membrane of the endoplasmic reticulum. Co-localisation of the TASK specific immunoreaction and the ER labelling was detected. The pixel analysis indicated 48 ± 12% co-localisation in the cases of TASK-1 subunits, while it was 25 ± 7% and 23 ± 11% in the cases of the TASK-2 and TASK-3 channel subunits, respectively (n = 5).

2.4. TASK-expression in the human cerebellum

The TASK expression pattern of the human cerebellum was also investigated in the present study. In the case of the human cerebellum the TASK-3 specific reaction was the strongest, although the TASK-1 specific reaction gave intense labelling too. The TASK-1 and TASK-3 expressions of the Purkinje-cells and pia mater were particularly strong. The TASK-2 expression was negligible, only the Purkinje- and granule cells showed weak positivity.

The results of the GFAP and TASK specific double labelling demonstrated that (similarly to the results obtained in the rat central nervous system) human cerebellar astrocytes also expressed TASK-1 and TASK-2 channels.

DISCUSSION

Significance of the HCN current of the pyramidal cells of the dorsal cochlear nucleus in shaping their characteristic activity pattern

The pyramidal neurones of the dorsal cochlear nucleus produced a slowly activating current on hyperpolarization without apparent inactivation tendency. The electrophysiological features of this current component, especially the rather positive reversal potential (-32 mV) indicated that the activity of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels were responsible for its genesis. The hyperpolarization-activated current of the pyramidal cells could be effectively blocked by extracellular application of 1 mM CsCl and by ZD7288, a specific blocker of the HCN channels, further confirming the identity of the channels.

Investigations of the electrical features of certain cardiac cells as well as some photoreceptors shed light on the presence and the activity of the current produced by the activation of HCN channels (formerly known as h-type current). It has been noted that this conductance is particularly important in providing the ability of spontaneous firing in certain cells. These channels activate close to the resting membrane potential, and allow the passage of a depolarizing mixed cationic current, which subsequently results in the depolarization of the cell membrane leading to action potential firing. Although this function of the HCN channels is particularly important in the pacemaker system of the heart, there are several data in the literature indicating that these channels are present in the surface membrane of certain neurones (e.g. thalamic relay neurones), providing the ability of spontaneous firing for these nerve cells. The HCN channels are also important in setting the resting membrane potential of the cells expressing them, and they are also involved in the regulation of their excitability. The presence and activity of the HCN channels can prevent long-term inhibition of the neurones possessing them.

Numerous reports indicated that different structures of the auditory pathway possessed HCN channels. The presence of the hyperpolarization-activated non-specific cationic current was proved on inner hair cells, on type I spiral ganglion neurones, on bushy neurones of the cochlear nucleus, on principal cells of the medial nucleus of the trapezoid body and on neurones of the superior olive. In the present work we added to the list one of the most important projection neurones of the dorsal cochlear nucleus, namely the pyramidal cell.

It has been known for a long time that after inserting microelectrodes into the dorsal cochlear nucleus, action potentials can be recorded even after the complete deafferentation of

this structure. The source of the spontaneous activity, however, has not been established yet. The present work proves that the pyramidal cells situated in isolated brain slice preparations possess spontaneous activity. The frequency of the spontaneous action potential firing was reduced to some 25% of the control activity in the presence of the usual blockers of the HCN channels (CsCl and ZD7288). We concluded, therefore, that the source of previously noticed spontaneous activity of the dorsal cochlear nucleus is (at least partially) the result of the intrinsic activity of pyramidal neurones, which, in turn, is maintained by the activity of their HCN-channels. If this assumption is accepted, two interesting questions can be formulated:

1. What might be the source of the ZD7288- and CsCl-resistant activity of pyramidal neurones (which is some 25% of their total activity)?
2. What might be the reason of that fact, that other structures of the auditory pathway, which certainly possess HCN current too, are not able to produce intrinsic firing (like bushy neurones)?

Although more experimental work is required to properly answer these questions, it seems to be likely that not only the pyramidal cells are capable of producing spontaneous activity in the cochlear nucleus complex, but some other structures (mostly certain interneurones) might be able to produce spontaneous activity. These neurones may be situated deeper in the slice; hence they are less sensitive to the HCN channel blockers. These cells, in turn, can activate the pyramidal cells via their synaptic connections. This theory is in accordance with those opinions that the granule cells of cochlear nucleus might also contribute to the spontaneous activity of the cochlear nucleus.

The key to the second problem may be the determination of the molecular structure of the pyramidal HCN channels, which was performed by using immunocytochemistry in the present study. HCN channels are formed by the homo- or heterotetrameric assembly of four different subunits (HCN1-HCN4). It is known that the homotetrameric HCN channels have markedly different electrophysiological properties: channels containing HCN1 subunits only activate rapidly (activation time constant < 100 ms), while channels containing HCN3 and HCN4 are much slower. The electrophysiological properties of the HCN-channels expressed by pyramidal neurones of cochlear nucleus (especially the activation kinetics of the hyperpolarization-activated current) suggested, that these channels could not be HCN1 homotetramers. Our immunohistochemical data supported this hypothesis, and showed that all investigated types of HCN subunits were present (HCN1, HCN2 and HCN4). These data about the presence of the indicated HCN subunits are in prefect accordance with earlier observations noting the presence of HCN1, HCN2, and HCN4 subunit specific mRNA in the

cochlear nucleus. On the bases of the results of the present work it cannot be excluded that the pyramidal neurones might possess HCN2/HCN4 heterotetrameric channels, similarly to the thalamic relay neurones. As thalamic relay neurones also possess the ability of spontaneous action potential firing, it is a tempting assumption that the HCN2/HCN4 subunit composition of neuronal structures might be responsible for ensuring the intrinsic activity. In the light of this suggestion we cannot exclude the possibility that other cells of the auditory pathway do not show spontaneous firing, because they lack HCN2/HCN4 heterotetrameric channels. Confirming this hypothesis must be the task of the not too distant future.

Investigation of presence and distribution of TASK-channels in human and rat central nervous system

On the basis of their molecular structure, K^+ channels belong to three superfamilies. Members of the voltage-gated K^+ channels (Kv-superfamily) are composed of four subunits, each containing six transmembrane domains, and a single pore forming loop. The subunits of the inward rectifier K^+ channels (Kir- superfamily) possess only those two transmembrane domains which are adjacent to the pore forming loop. Similarly to the Kv channels, the assembly of the inward rectifier K^+ channels requires four individual subunits. The most recently discovered superfamily of K^+ channels is has entirely different molecular structure. The channel forming subunits have 4 transmembrane domains, and they possess two pore-forming loops in tandem. To form a functional channel, two subunits must bind to each other (dimerisation). As the consequence of the highly unique molecular structure, this group of K^+ channels is usually referred as 4TM-PP or twin-pore-domain channels.

The twin-pore-domain K^+ channel superfamily consists of numerous individual members. TASK channels, known to be very sensitive to the alterations of the extracellular pH, seem to be particularly important. TASK channels have ubiquitous distribution, and they are supposed to have crucial roles in setting the resting K^+ conductance of the cells, and (consequently) they regulate the resting membrane potential of both excitable and non-excitable cells, along with the excitability of the excitable structures. In fact, TASK channels are further divided into two subgroups, as the TASK-1, TASK-3 and TASK-5 channels show more significant sequence homology with each other, than with TASK-2 and TASK-4. The latter two channels are nowadays regarded as members of a completely different subgroup (termed TALK family).

Previous studies indicated very strong TASK-specific mRNA expression in different regions of the auditory pathway (including the cochlear nucleus), but hitherto no investigations have been conducted to check whether TASK channels were present at the protein level too. Two more interesting facts caught our attention prior to commencing the present experiments: there were indications that the TASK channels might be expressed by astrocytes; and (besides an investigation regarding the presence of TASK specific mRNA in crude extracts originating from human tissue samples) there were effectively no useful data about the possible distribution of TASK-channels in the human central nervous system. One of the purposes of the present work was to compare the TASK distribution in the rat and human central nervous system, hence the cerebellum was employed, as its histological structure is relatively simple, and it has a close developmental relationship with the dorsal cochlear nucleus.

One of our most important experimental findings was the realisation that the distribution of the TASK channels is not identical in the rat and human cerebellum. While in the rat cerebellum the TASK-1 specific immunoreaction was the strongest and TASK-3 specific staining was the weakest, in the human cerebellum the TASK-3 specific expression was the most significant. TASK-1 specific reaction could also be noticed (although it was weaker than the TASK-3 expression), while the TASK-2 specific reaction was almost absent, only Purkinje-cells showed detectable (but fairly weak) staining. Our observations were in good accordance with the results of another study performed on crude cerebellar extract, detecting the presence of TASK specific mRNA. In the human cerebellum the Purkinje-cells and pia mater showed particularly strong TASK-3 and somewhat weaker TASK-1 positivity. By using double immunolabelling TASK-1 and TASK-3 expression could be indicated on human cerebellar astrocytes too.

The TASK-expression of the astrocytes was further investigated in astrocyte cultures prepared from different regions of the rat brain (cerebellum, hippocampus, cochlear nucleus). Our results indicated that although all of the investigated TASK channel types were expressed by astrocytes isolated from the rat brain (the TASK-1 positivity was the strongest, the TASK-2 and TASK-3 specific immunoreactions were somewhat weaker), the two main types of astrocytes, namely GluT and GluR types showed strong difference in terms of the TASK expression: while GluT cells, characterised by having relatively small soma and numerous thin processes showed intense TASK-1 immunolabelling, GluR cells (possessing bigger cell body and fewer but thicker processes) demonstrated weaker TASK-1 specific immunoreaction.

The activity of the TASK channels could be proved by electrophysiological measurements performed on both types of astrocytes. Although further investigations are required to determine the specific importance of the glial TASK channels, it can be assumed that the K^+ movement through these channels might be involved in compensating for the alterations of K^+ concentrations accompanying neuronal activity.

In our experiments a fine, network-like TASK immunolabelling could be demonstrated in astrocytes. The confocal microscopy data clearly indicated that it corresponded to the TASK positivity of certain intracellular structures. Further experiments confirmed that a certain portion of TASK channels was situated in the membrane of the endoplasmic reticulum. Interestingly, there are data about the movement of TASK-1 channels between the surface membrane and the endoplasmic reticulum in TASK-1 specific DNA transfected COS-cells. Our data indicate that this movement of the TASK-channels (which may be responsible for rapidly and effectively modifying the TASK density in the surface membrane) may also be present under physiological conditions (in non-transfected cells) too.

Publications providing the basis of the present theses

Papers

1. Pál, B., Pór, Á., Szűcs, G., Kovács, I., Rusznák, Z. (2003) HCN channels contribute to the intrinsic activity of cochlear pyramidal cells. Cell. Mol. Life Sci. 60: 2189-2199. **(IF: 5,259)**
2. Rusznák, Z., Pocsai, K., Kovács, I., Pór, Á., Pál, B., Bíró, T., Szűcs, G. (2004) Differential distribution of TASK-1, TASK-2 and TASK-3 immunoreactivities in the rat and human cerebellum. Cell. Mol. Life Sci. (közlésre elfogadva, nyomtatás alatt) **(IF: 5,259)**

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5. Rusznák, Z., Pál, B., Pór, Á., Pocsai, K., Szűcs, G. (2003) Characterisation and function of an h-current presented by the cochlear pyramidal cells of the rat, 3rd FEPS Congress, Nice, France.
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1. Pál, B., Pór, Á., Pocsai, K., Szűcs, G., Rusznák, Z. Voltage-gated and background K^+ channel subunits expressed by the bushy cells of the rat ventral cochlear nucleus. Hearing Research (bírálatra elküldve)
2. Pál, B., Rusznák, Z., Harasztosi, Cs., Szűcs, G. Depolarization-activated K^+ currents of the bushy neurones of the rat cochlear nucleus in a thin brain slice preparation. Acta Physiologica Hungarica (bírálatra elküldve)

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8. Pór, Á., Pál, B., Rusznák, Z., Szűcs, G. (2003) Immunocytochemical investigation of the ionic channels expressed by neurones of the rat cochlear nucleus, XXXIII. Membrán-Transzport Konferencia, Sümeg.